EFFECT OF LOW-, MODERATE-, AND HIGH-INTENSITY EXERCISE ON ATRIAL NATRIURETIC PEPTIDE (ANP) AND SARCOPLASMIC/ENDOPLASMIC RETICULUM CALCIUM-ATPase 2a (SERCA2a) GENE EXPRESSION IN WISTAR RAT CARDIAC MUSCLE

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Abstract

Exercise is one of the factors that affect cardiovascular health. Exercise has been proven to reduce the number of cardiovascular diseases. However, it is also known that doing intense exercise has an adverse effect on heart health which can increase the risk of cardiovascular disease. While doing exercise, there is compensation from the heart itself by producing substances to maintain the heart to remain functional, known as cardiac biomarkers, including ANP and SERCA2a. Thus far, the recommended intensity is moderate-intensity exercise, which is optimal for heart health, but this recommendation has not been supported by biomolecular study. Therefore, this research was conducted. This study was done using semi-quantitative analytic method and experimental study design. Total of 24 male wistar rats were divided randomly into 4 groups: 1 control group (0m/min) and 3 exercise groups (low-: 10m/min, moderate-: 20m/min, high- intensity exercise: 30m/min). All groups received 30 min/day running, 5x/week for 12 weeks. The rats were terminated under anaesthesia, cardiac muscle extraction was done from left ventricle, and gene expression of ANP and SERCA2a is studied. Statistical analysis was done using SPSS software with one-way ANOVA followed by post hoc comparisons if the p-values < 0.05. The expression of ANP increased but insignificant in low-, moderate-, and high-intensity exercise group of rat (0.872 ± 0.034 vs 0.901 ± 0.018 vs 0.916 ± 0.015 vs 0.938 ± 0.025; p=0.304). The expression of SERCA2a also increased insignificantly in low-, moderate-, and high-intensity exercise group of rat (1.044 ± 0.019 vs 1.051 ± 0.015 vs 1.063 ± 0.011 vs 1.082 ± 0.027; p=0.493). Different exercise intensity didn't have significant effect on ANP and SERCA2a expression in rat cardiac muscle.

Keywords: cardiac, exercise, intensity, ANP, SERCA

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INTRODUCTION

According to WHO, cardiovascular disease (CVD) is a major cause of death in the world[1]. The risk factors of CVD include high calorie diet, lack of physical activity, smoking, cholesterol, hypertension, and others. Lack of physical activity is the fourth highest risk factor that enhance the risk of death in the world after high blood pressure, smoking, and high blood glucose[1-3]. Studies show that exercise can decrease the risks of cardiovascular disease. Regular exercise has important roles in improving the cardiac health and preventing the cardiovascular disease[1,4-6]. However, the intense exercise also can cause adverse effects on the cardiovascular itself, the heart and large blood vessels undergo pathological structure remodeling that actually increase the risk of cardiovascular disease[4].

Exercise is useful for improving physical fitness and enhances the cardiac function[5]. Exercise at particular intensity alter the components of Atrial Natriuretic Peptide (ANP) and Sarcoplasmic/Endoplasmic Reticulum Calcium-ATPase (SERCA2a) as the biomarkers for cardiac function. ANP is natriuretic peptide that released from heart when atrium and ventricle distention by neuro humoral stimuli, commonly caused by exercise[7, 8]. It also acts as an indicator of the functionality degree of cardiac function in patients with cardiac disease[9]. The increase of this hormone is associated with better survival in patients with chronic heart failure (CHF). The worse the progression of heart failure, ANP will also fail to compensate[7, 8]. Cardiac SERCA2a plays a central role in myocardial contractility. Decreases in the expression levels of SERCA2a have been observed in a variety of pathological conditions. Thus, SERCA2a is a major regulator of intracellular Ca^{2+} homeostasis, and changes in the expression and activity of the SERCA pump contribute to the decreased SERCA2a content and cardiac dysfunction during pathogenesis[10, 11].

The intensity of exercise will affect the energy used. Various researches have studied about the various benefits of exercise, especially on molecular and cellular mechanisms in the body. However, future researches are needed to determine the optimal intensity of exercise[12,13]. Thus far, moderate-intensity exercise is recommended as the optimal intensity of exercise for improving the physical fitness and preventing the cardiovascular disease[3,14]. However, there is no study about the effect of the different exercise intensity on ANP and SERCA2a expression in rat cardiac muscle[8,14]. Therefore, this study was conducted to identify the cardiac function through the change in ANP and SERCA2a expression in rat cardiac muscle that received exercise at low-, moderate-, or high-intensity.

MATERIAL AND METHODS
Animals

All the animal experiment procedures in this study were approved by Health Research Ethics Committee Faculty of Medicine Universitas Padjadjaran No 986/UN6.KEP/EC/2018 and also followed Guide for the Care and Use of Laboratory Animal (The National Academic press, 2011). Total of 24 male wistar rats (Biofarma Laboratory, Bandung, Indonesia) at the age of 8 weeks with body weight 201-250 gram used in the present study. Every 5 rats were kept in a cage (40 cm x 70 cm x 40cm) with food and water available *ad libitum*. Animals were kept under a 12 h light-12 h dark cycle and room temperature. After done the treadmill habituation for 2 weeks, the rats were divided randomly into four groups: control group (0m/min) and 3 exercise groups low-: 10m/min, moderate-: 20m/min, high-intensity: 30m/min)[15].

Treadmill apparatus and training protocol

The authors used animal treadmill machine (IDEAS, Bandung, Indonesia) for rat. The treadmill machine is divided into five lanes. All rats were habituated for two weeks by placing rats in the running treadmill machine at a speed that gradually increase every three days. At the end of habituation, rats were divided randomly into four groups (control, low-, moderate-, high-intensity). The

rats were run in individual lane according to the group (control: 0 m/min, low-: 10 m/min, moderate-: 20 m/min, high-intensity: 30 m/min)[15]. All groups were run for 30 min/day, 5x/week for 12 weeks.

Measurement of ANP and SERCA2a gene expression

By the end of training period, all rat groups (control and exercise) were terminated under inhaled isoflurane anesthesia and then the hearts were isolated. The hearts were kept in eppendorf tubes, snap-frozen in liquid-nitrogen then stored at -80°C until RNA isolation.

Total RNA were extracted from the cardiac muscle using TRIzol reagent (Thermo Fisher Scientific, Carlsbad, CA). Semi-quantitative PCR was performed using the RT PCR Kit (Bioline, London., UK). The gel electrophoresis band of the PCR products of each gene was visualized by BioDocAnalyze gel analysis software and quantified using ImageJ Software (NIH). The gene expression level of the samples was normalized by GAPDH mRNA level. The sequences and other information about the primers including ANP, SERCA2a, and GAPDH are listed in Table 1.

Table 1. Primers for real-time polymerase chain reaction

Gene	Sequence	Tm(°C)	Product (bp)
ANP	F 5'- ACC TGC TAG ACC ACC TGG AG -3' R 5'- AGC CCT CAG TTT GCT TTT CA -3'	61	227
SERCA2a	F 5'- ATT GTT CGA AGT CTG CCT TCT GTG G -3' R 5'- CAT AGG TTG ATC CAG TTA TGG TAA A -3'	64	175
GAPDH	F 5'- GTTACCAGGGCTGCCTTCTC -3' R 5'- GATGGTGATGGGTTTCCCGT -3'	61	177

*Tm : temperature melting
**Bp : base product

Statistical analysis

All statistics were computed using SPSS version 25.0 software for Windows. Quantitative data were expressed as the means ± standard error of mean (means ± SEM). Data were statistically analyzed with one-way ANOVA followed by post hoc comparisons using Dunnett. The p-values < 0.05 were considered to be significant.

RESULT

The ANP expression level in control group was 0.872 ± 0.034, low-intensity group was 0.901 ± 0.018, moderate-intensity group was 0.916 ± 0.015 and high-intensity group was 0.938 ± 0.025. ANP mRNA expression in low-intensity group increased compared with the control group although statistically not significant, also in moderate- and high-intensity group, that ANP expression increased but statistically not significant. The results showed that all exercise intensity group didn't have significant effect on ANP mRNA expression ($p = 0.304$).

The SERCA2a expression level in control group was 1.044 ± 0.019, low-intensity group was 1.051 ± 0.015, moderate-intensity group was 1.063 ± 0.011 and high-intensity group was 1.082 ± 0.027. SERCA2a mRNA expression in low-intensity group increased compared with the control group although statistically not significant, same as in moderate- and high-intensity group, SERCA2a expression also increased insignificantly compared with the control group. The results showed that all exercise intensity group didn't have significant effect on SERCA2a mRNA expression ($p = 0.493$).
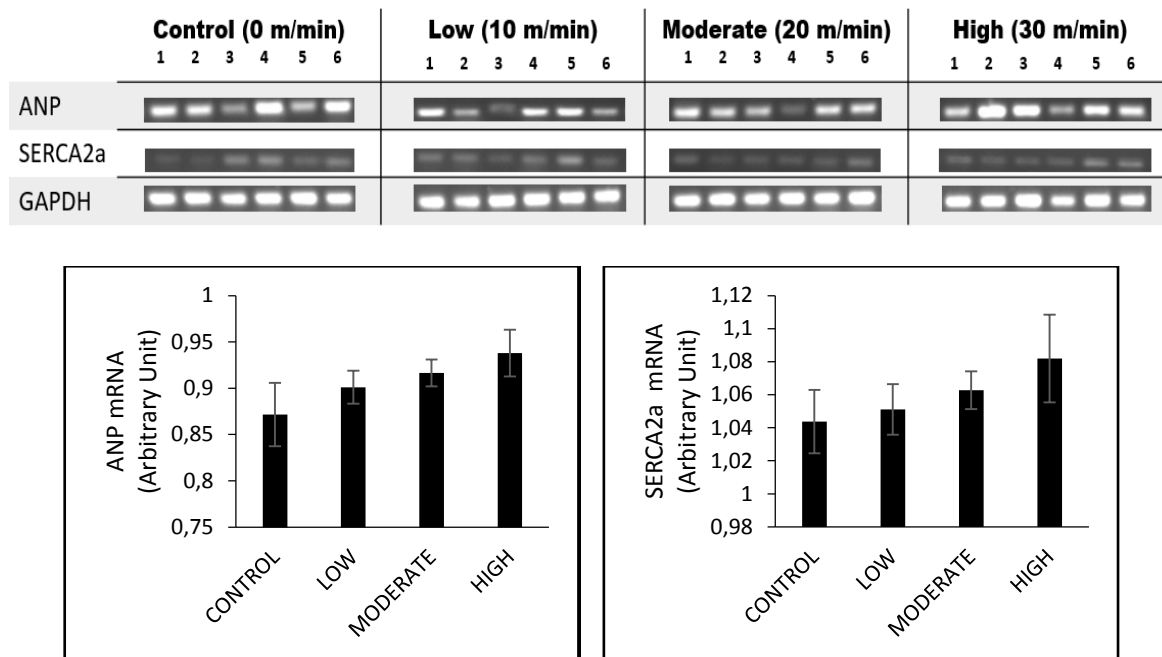
Figure 1. PCR Result of ANP and SERCA2a gene expression.
Values are expressed as mean ± SEM.

DISCUSSION

Exercise influences the body homeostasis thus can cause the cardiovascular physiologic response. The needs of myocardial metabolic will increase during physical exercise and cause increasing myocardial oxygen consumption. When the heart rate increases, myocardial oxygen consumption increases, so that the blood flows will also increase through two mechanisms: (1)Increasing myocardial contraction due to response to exercise increase the blood flow to the coronary circulation. (2)Vasodilation of arterioles that supply the myocardium will increase the blood flow and decrease the vascular resistance. Increased heart rate is influenced by the intensity of exercise therefore the intensity of exercise will also affect myocardial needs including myocardial oxygen consumption[14]. During exercise, adaptations occur to meet the oxygen demand in myocardium. Therefore, cardiac myocytes undergo an increase in oxidative stress in the body after doing exercise. However, the threshold of the intensity that starts to show an increase in oxidative stress levels is still unknown yet[16]. The chronic response to exercise is obtained after individuals doing the exercise regularly for 8 to 12 weeks[17].

The studies of patients with congestive heart failure (CHF) showed the increased concentration of ANP in response to exercise. Exercise is accepted as a strong stimulus for the release of ANP. The pro-ANP levels increased gradually after doing exercises, these findings may represent a beneficial effect of increasing the capacity of ANP in CHF patients after completion of exercise.[18] Recent study also showed there was the increase of mRNA ANP levels in hamster cardiac muscle with CHF[19].

In healthy humans, it has been shown that ANP increase during and immediately following the exercise. ANP levels increased significantly in subjects who did the exercise. ANP increased rapidly after 5-10 minutes of exercise. A study conducted by Follenius *et al*. showed a rapid increase in plasma ANP levels was induced by both high (75% VO_2max) and lower work levels (55% VO_2max)[20]. In Follenius *et al*. research also has the similar result with our study which the increase of ANP level according to the intensity of exercise were not significantly different in either case[20].

In study conducted by Haufe *et al*. with overweight and obese subjects were measured the pro-ANP levels in rest and after exhaustive exercise using a bicycle ergometer

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until exhaustion[21]. The study showed that after six months weight loss and exercise, the pro-ANP levels did not change, however, the acute measurement showed increased in pro-ANP levels during exercise both before and after dietary intervention. This study concluded that acute exercise significantly increased the pro-ANP levels in overweight and obese patients, but chronically has no effect and there could be dietary influencing[21].

Study from Bordbar *et al.* analyze the effects of eight weeks training exercise on the natriuretic peptide release[22]. This study showed that natriuretic peptide significantly increased immediately after aerobic exercise, but after eight weeks of exercise it decreased. The result showed that in chronic exercise, natriuretic peptide levels return to baseline[22]. Possibly, in chronic exercise, the adaptation process already occurred, therefore can cause the lower level of ANP expression as in this study.

SERCA2a regulates the transport of Ca^{2+} out of the cytosol into the sarcoplasmic reticulum just after the myocardial contraction has occurred. The increase of SERCA2a in response to exercise could be an adaptation to the heart to prevent any dysfunction[23]. In this study, SERCA2a mRNA expression increased in exercise group although the different level of intensity among the group was statistically insignificant. Similarly in a study on the heart of obese rat that do running also showed the increase in SERCA2 mRNA levels, suggesting that regular exercise has a beneficial effect on cardiac function[24].

Study that analyzes the effect of different exercise intensity on SERCA2a gene expression is a quite rare until now. Kubo *et al.* conducted the study in Sprague-Dawley rats trained 60 min per day, 5 days per week for six weeks at moderate- and high-intensity showed significantly increased than control in SERCA2a mRNA expression on gastrocnemius muscle after exercise for both intensity groups[25]. Similarly as study from Wisloff *et al.* in infarct Sprague-Dawley rats which ran on a treadmill 1.5 hours per day, 5 days per week for eight weeks showed that

aerobic endurance training significantly increased the myocardial SERCA2[26]. Study from Medeiros *et al.* male wild-type (WT) mice that performed swimming 5 days per week for eight weeks also showed a significant increase in SERCA2 expression.[27] Possibly, the duration of exercise in this study was shorter compared with previous papers (30 min vs 60-90 min) cause the lower level of SERCA2a expression[25,26].

CONCLUSION

The expression of ANP and SERCA2a in rat cardiac muscle were not affected by the different exercise intensity. Further experiments are needed to analyze the correlation between ANP and SERCA2a. Also the histopathology or immunohistochemistry examination of rat cardiac muscle need to conduct for further analysis which exercise intensity is the most optimal for cardiovascular health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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